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Renal toxicity of phosphate in rats

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Renal toxicity of phosphate in rats. To evaluate the mechanism by which phosphate induces renal injury, we placed uninephrectomized, partially nephrectomized, and intact rats on dietary phosphorus intakes varying between 0.5 and 2% for 18 weeks. None of the animals on a normal phosphorus intake (0.5%) had any abnormalities. Four out of six intact animals on a 1% phosphorus diet had kidney calcium concentrations within the normal range, and only one showed any histologic changes. In contrast, all but one partial and uninephrectomized animals on a 1% phosphorus diet had increased kidney calcium content concentration, and five of the six studied had histologic changes. The degree of calcification and histologic changes in the uninephrectomized animals on a 1% phosphorus diet was similar to that found in the intact animals on a 2% phosphorus diet. Animals on a 3% phosphorus diet plus disodium ethane-1-hydroxy-1-1-diphosphonate (EHDP) had significantly less calcification and histologic changes than did animals on a similar diet without EHDP. *Conclusion.* As renal functional mass is reduced, the nephrotoxicity of phosphorus is greatly enhanced. Phosphorus-induced renal injury is mediated through calcium phosphate deposition in the kidney. This results from intrarenal causes, because the kidney calcification can be related to phosphate excreted per functional unit rather than plasma phosphate concentrations.

La toxicité rénale du phosphate chez le rat. Pour évaluer le mécanisme par lequel le phosphate détermine des lésions rénales, des rats uninéphrectomisés, partiellement néphrectomisés, et intacts ont reçu des apports de phosphate alimentaire divers compris entre 0,5 et 2% pendant 18 semaines. Aucun des animaux soumis à un apport de phosphorus normal (0,5%) n'a eu d'anomalie. Quatre parmi les six animaux intacts soumis à un apport de 1% avaient des concentrations rénales de calcium comprises dans l'éventail des valeurs normales et seulement un d'entre eux avait des lésions histologiques. Au contraire, les animaux partiellement néphrectomisés ou uninéphrectomisés soumis à un apport de 1% de phosphorus avaient tous, sauf un, une augmentation du contenu rénal en calcium. Cinq parmi les six étudiés du point de vue histologique avaient des lésions. L'importance de la calcification et des modifications histologiques chez les animaux uninéphrectomisés soumis à un apport de 1% de phosphorus était comparable à celle observée chez les animaux intacts soumis à un apport de 2% de phosphorus. Les animaux soumis à un apport de 3% de phosphorus et à le disodium ethane-1-hydroxy-1-1-diphosphonate (EHDP) avaient significativement moins de calcification et de modifications histologiques que les animaux recevant la même alimentation sans EHDP. *Conclusion.* La réduction de la masse fonctionnelle rénale augmente considérablement la toxicité du phosphore. L'atteinte rénale liée au phosphore a pour médiateur la déposition de calcium phosphate dans le rein. Cela est la conséquence d'un mécanisme intra-rénal puisque la calcification est en rapport avec l'excrétion de phosphore par unité fonctionnelle plutôt qu'avec la concentration plasmatique de phosphore.

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Several reports document the injurious effect of high-phosphate intake on the kidney [1–4]. Largely, it is assumed that calcium phosphate deposition resulting from hyperphosphatemia incites an inflammatory and fibrotic response in a variety of organs, explaining the mechanism of tissue injury [5, 6]. A number of findings, however, suggest that the pathogenesis of renal injury from a high-phosphate diet may be different. First, the histologic appearance of the calcium phosphate deposits suggests they result from intrarenal mechanisms [2]. Second, the kidney calcifies early during the course of renal failure, prior to the time that plasma phosphorus concentrations are appreciably changed, whereas other organs calcify in the terminal phase of renal failure in association with markedly increased plasma phosphorus concentrations [7, 8].

Phosphate-induced renal damage has assumed increasing importance in view of the recent studies that show that a phosphate-restricted diet fed to animals with a remnant kidney [9] or experimental glomerulonephritis [10] prevents calcification, functional deterioration, and histologic changes.

The mechanism by which phosphate restriction prevents progressive destruction of the diseased or damaged kidney has not been defined. It is known, however, that with both increased phosphorus intakes [11–13] and reduction of functional renal mass [14] that adaptive mechanisms are brought into

play, which are not necessarily dependent on PTH, to maintain phosphate balance. It seems possible that this adaptive mechanism that results in an increased phosphate excretion per nephron could be responsible for the calcium phosphate deposition and renal injury.

The purpose of this study was to test whether increased renal excretion of phosphorus could induce renal calcification and injury in the absence of a rise in plasma phosphorus concentration. By surgical procedures, we removed various fractions of the renal mass and nephron population. We then gave the animals normal to elevated phosphate intake, so as to widely vary phosphate load excreted per nephron without changing plasma phosphate concentrations. To determine if phosphate renal injury was mediated through calcification or phosphate loads themselves, we gave the animals diphosphonates to decrease the severity of the calcium phosphate deposits while maintaining a high renal phosphate load.

Methods

Fifty-four male Sprague Dawley rats (Simonson Labs, Gilroy, California), each weighing between 200 and 250 g, were divided into three groups (1, 2, and 3) of 18 animals each. Each group was evenly divided into three subgroups: A, B, and C. Animals in subgroup A were left intact. Animals in subgroups B (partial nephrectomy) had the outer poles of the left kidney removed by ligation and cautery as previously described [9], but the contralateral kidney was left intact. Animals in subgroup C (uninephrectomy) underwent a left nephrectomy. All operations were performed through a flank incision, after the rats were anesthetized with ether. Immediately postoperatively, the animals were begun on their experimental diets (vide infra).

They were fed a synthetic low-phosphorus diet (ICN Pharmaceuticals Incorporated, Cleveland, Ohio) to which a balanced phosphorus mixture (four parts monobasic sodium phosphate; one part diabasic sodium phosphate) was added to make a 0.5% phosphorus diet (group 1), a 1.0% phosphorus diet (group 2), and a 2% phosphorus diet (group 3). The diet was fully fortified with vitamins and contained 20% protein (bovine fibrin). On analysis, it contained 0.56% calcium, 0.02% magnesium, and 0.04% phosphorus before supplementation. Throughout the study period, animals in all groups received and consumed an equal amount of food (approximately 10 g/day).

Immediately prior to surgery and to beginning the experimental diets, we drew control blood samples for creatinine, calcium, and phosphorus by percutaneous cannulation of the tail artery. After beginning the experiment we obtained blood samples for chemical analysis at 3-week intervals.

At 7 weeks into the study, four animals from each group were placed overnight into individual stainless steel wire mesh metabolic cages. Starting the next morning, we gathered two consecutive 24-hour urine collections for measurement of volume, creatinine, calcium, and phosphorus. Each animal was given 10 g of food each day, which was always fully consumed. Blood samples were drawn from the animals upon completion of the urine collections.

At 18 weeks into the study, the animals were weighed and lightly anesthetized with ether, and their blood pressures were directly measured by cannulating their abdominal aortas with a 20-gauge needle connected to a transducer (Statham model-P23D, Statham Medical Instruments, Inc., Hato Rey, Puerto Rico). The pressures were recorded on a recorder (Model 7702B, Hewlett-Packard, Waltham, Massachusetts). Next, the animals were exsanguinated via the aortic needle and the blood was collected for chemical analysis. An additional 18 rats were killed after they had received the diet for 10 weeks. Six intact animals and six uninephrectomized animals had received the 1% phosphorus diet, and six intact animals had ingested the 2% phosphorus diet for this 10-week period. The kidneys were removed and weighed, and a coronal section of each kidney was immediately fixed in 10% buffered formalin for histology. The remainder of the kidney tissue, as well as a section of the aorta, was dried and analyzed for calcium content by previously reported techniques [5].

Serum and urine samples were analyzed on an autoanalyzer (Technicon II) for creatinine [15]. Phosphorus was measured with an automated modification of the method of Fiske and Subbarow [16], and calcium was measured with an atomic absorption spectrophotometer (Perkin Elmer, model 290B), as previously described [5].

Kidney sections were stained with hematoxylin and eosin. Slides from the intact and nephrectomized groups were coded and given to an experienced renal pathologist, who read the slides without knowledge of the code. The slides were evaluated for histologic severity of disease from zero (no abnormality) to 3+ (marked abnormality). The *histologic severity index* was the mean grade of the fol-

lowing parameters, each of which was individually scored: interstitial edema, interstitial infiltrate, interstitial fibrosis, tubular atrophy, and tubular dilatation.

Statistical comparisons were performed with unpaired Student's *t* test, except where otherwise stated. All values are expressed as the means ± SEM.

Diphosphonate study. Ten male Sprague Dawley rats, each weighing between 275 and 325 g, were evenly divided into two groups (A and B). While receiving standard Purina rat chow, the animals in group A were given a daily i.p. injection of disodium ethane-1-hydroxy-1-1-diphosphonate (EHDP) in saline (supplied by Procter and Gamble) at a dose of 5 mg/kg for 10 days.

After the initial 10 days, the animals in both groups were placed on the experimental diet. The experimental diet was finely ground Purina rat chow, to which the balanced phosphorus mixture (vide supra) was added to make a 3% phosphorus diet. The diet was fully fortified with vitamins and contained 24% protein. On analysis, it contained 1.5% calcium, 0.2% magnesium, and 0.5% phosphorus before supplementation.

The animals were fed the experimental diet twice daily, at 8:00 A.M. and 4:00 P.M. Each feeding consisted of 10 g/rat. Immediately prior to each feeding, the five animals in group A each received an i.p. injection of EHDP in saline for a total daily dose of 10 mg/kg. Whether or not the food was completely consumed, the food dishes were removed from the cage after 4 hours at each feeding. This method of feeding was carried out to insure that the animals had maximum urine and plasma diphosphonate concentrations during the time they were ingesting phosphate.

Blood samples were obtained for creatinine and phosphorus by tail artery cannulation at the beginning of the study and at 1 to 2-week intervals thereafter. At 1 week and at 5 weeks after starting the 3% phosphorus diets, the animals were placed in metabolic cages overnight, and starting the next morning they had two consecutive 24-hour urine collections for measurement of volume, creatinine, and phosphorus.

At 6 weeks, both groups were bled for chemical analysis, and all animals underwent a left nephrectomy under ether anesthesia. The kidneys were weighed, and a coronal section of each was immediately fixed in 10% buffered formalin for histology. The remainder of the kidney was dried and analyzed for calcium content (vide supra).

Results

The study was designed to end when one or more groups developed uremia, as defined by a plasma creatinine concentration greater than 1 mg/dl. This occurred at 18 weeks.

The results of the metabolic studies carried out at week 7 are shown in Table 1. Urinary phosphorus excretion reflected the phosphorus intake in all groups of animals. Phosphorus excretion was similar in animals maintained on a similar phosphorus intake, whether they were intact, partially nephrectomized, or uninephrectomized. Considerable functional hypertrophy had occurred as evidenced by the fact that the mean creatinine clearance was only slightly less in the uninephrectomized and partially

Table 1. Seven-week balance study[a]

Group[b]	Dietary P *mg/day*	C_{Cr} *ml/min/100 g body wt*	Urinary P *mg/day*	P load excreted per nephron *μg/day*	Urinary Ca × P *(mg/dl)²*
Group 1 (0.5% P)					
A	50	0.55 ± 0.04	31.0 ± 1.3	0.52 ± 0.02	195 ± 36
B	50	0.57 ± 0.03	30.0 ± 2.1	—	163 ± 25
C	50	0.47 ± 0.03	31.0 ± 1.3	1.04 ± 0.04	283 ± 115
Group 2 (1.0% P)					
A	100	0.53 ± 0.08	72.0 ± 1.1	1.20 ± 0.02	354 ± 64
B	100	0.51 ± 0.05	64.0 ± 3.3	—	379 ± 38
C	100	0.49 ± 0.04	64.0 ± 6.5	2.14 ± 0.22	379 ± 79
Group 3 (2.0% P)					
A	200	0.48 ± 0.05	150.0 ± 19.4	2.51 ± 0.32	702 ± 84
B	200	0.37 ± 0.03	144.0 ± 9.8	—	498 ± 34
C	200	0.42 ± 0.03	152.0 ± 12.6	5.07 ± 0.42	742 ± 78

[a] Values are the means ± SEM.
[b] Subgroups are designated as A, intact kidneys; B, partial nephrectomy; C, uninephrectomy.

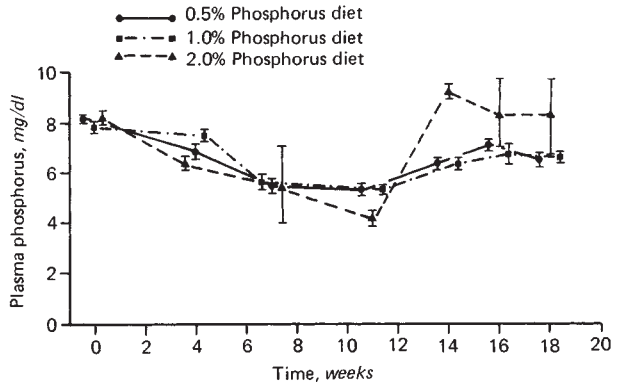

Fig. 1. *Serial plasma phosphorus concentrations in the intact rats in groups 1, 2, and 3.*

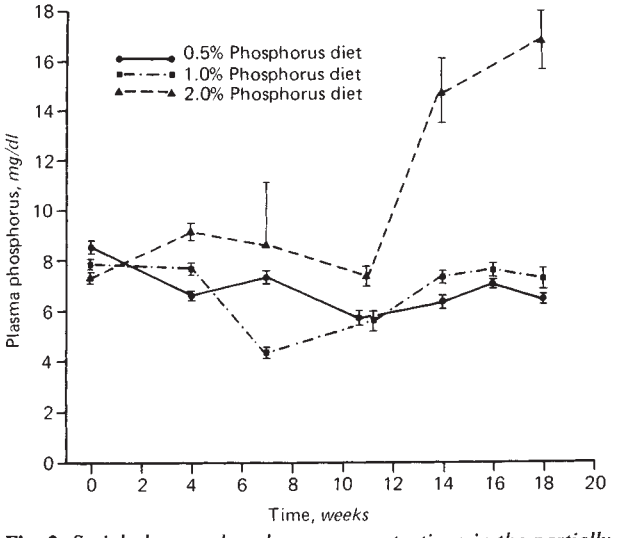

Fig. 2. *Serial plasma phosphorus concentrations in the partially nephrectomized rats in groups 1, 2, and 3.*

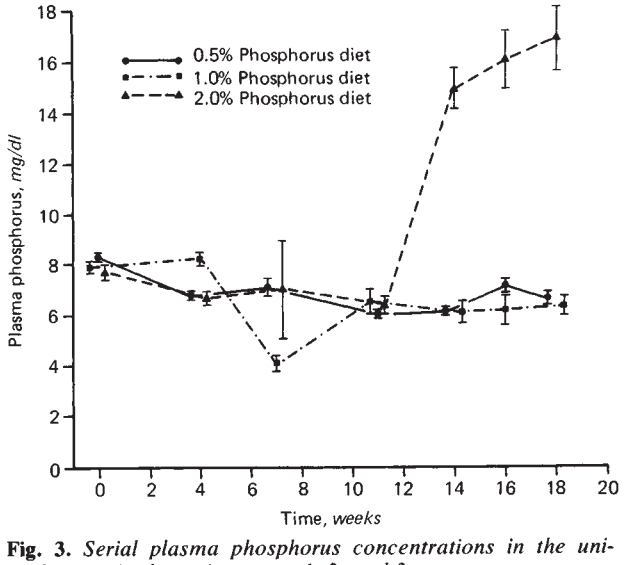

Fig. 3. *Serial plasma phosphorus concentrations in the uninephrectomized rats in groups 1, 2, and 3.*

nephrectomized groups than it was in the intact groups.

The average phosphorus load to be excreted per nephron was calculated, assuming 3×10^4 nephrons per rat kidney. This calculation was not performed for the partial nephrectomy groups, because the exact amount of renal tissue removed was unknown. As can be appreciated in Table 1, uninephrectomized animals on a 0.5% phosphorus diet had a phosphorus excretion per nephron similar to the intact animals on 1% phosphorus diet, and the uninephrectomized animals on a 1% phosphorus diet had a phosphorus excretion per nephron similar to intact animals on a 2% phosphorus diet. The urinary calcium-times-phosphorus products increased with every dietary increment of phosphorus, but showed little intragroup variation.

Serial plasma phosphorus concentrations obtained throughout the study are shown in Fig. 1 (intact animals), Fig. 2 (animals with partial nephrectomy), and Fig. 3 (animals with unilateral nephrectomy). Throughout the study period, plasma phosphorus concentrations were similar in the animals on 0.5% and 1% phosphorus diets in all the subgroups (Figs. 1 to 3). In the intact animals on 2% phosphorus, plasma phosphorus showed no consistent difference until 14 weeks, when the phosphorus increased and remained higher at 16 and 18 weeks, although the difference from the other groups fell short of significance. The same pattern was observed in the uninephrectomized and partially nephrectomized animals on the 2% phosphorus diet. These groups had a more marked elevation of phosphorus at 14 weeks, which persisted at significantly higher values ($P < 0.001$) than it did in the other groups at each subsequent determination (Figs. 2 and 3).

During the latter half of the study, plasma calcium concentrations were significantly less in animals with reduced renal mass on 2% phosphorus diet as compared with the other two groups. Animals on 0.5% and 1% phosphorus diet had similar calcium concentrations throughout the study period with the exception of the final values obtained at 18 weeks, which were 10.5 ± 0.08 in group 1 vs. 9.5 ± 0.15 in group 2 ($P < 0.001$) and 8.2 ± 0.65 mg/dl in group 3 ($P < 0.005$).

At 18 weeks, group 3C (2% phosphorus, one kidney) became uremic with a mean plasma creatinine concentration of 1.08 ± 0.12, which was significantly elevated ($P < 0.001$) as compared with the control group 1A (0.5% phosphorus with two kidneys) (0.48 ± 0.02). Group 3B (2% phosphorus, partial ne-

Table 2. Histologic and analytical data[a]

Group[b]	Histologic severity index	Quantity, *mmoles/kg DDFT*		Animal's final weight *g*
		Ca	P	
Group 1 (0.5% P)				
A	0	13.7 ± 1.2	335 ± 6	393 ± 7
B	—	13.7 ± 2.0	354 ± 12	389 ± 15
C	0	12.8 ± 1.7	359 ± 7	365 ± 12
Group 2 (1.0% P)				
A	0.1 ± 0.1	60 ± 37	360 ± 7	367 ± 12
B	—	147 ± 83	417 ± 37	327 ± 9
C	1.1 ± 0.3	589 ± 417	654 ± 222	342 ± 23
Group 3 (2.0% P)				
A	1.6 ± 0.1	431 ± 40	700 ± 154	365 ± 30
B	—	2124 ± 555	1457 ± 323	265 ± 22
C	2.6 ± 0.1	2252 ± 501	1393 ± 241	271 ± 10

[a] Values are the means ± SEM. DDFT is dry defatted tissue.
[b] Subgroups are defined in Table 1.

phrectomy) also had plasma creatinine concentrations (0.97 ± 0.14) that were significantly higher than those of group 1A ($P < 0.005$). At this time, mean plasma creatinine concentration in no other group was significantly different from that of group 1A. In addition, plasma creatinine in every animal of the other groups was within the normal range for our lab, except for one animal in group 2C (1% phosphorus, one kidney), which had a creatinine of 1.7 mg/dl at sacrifice.

There was no significant difference in blood pressure among the groups at sacrifice.

Table 2 includes the mean histologic severity index, kidney calcium and phosphorus content, and animal weight for each of the nine subgroups.

Animal weights were similar in all groups, except for the uninephrectomized and partially nephrectomized animals on the 2% phosphorus diet, which were lower than the others (Table 2).

All animals in group 1 (0.5% phosphorus) had similar kidney calcium concentrations, which were in the normal range for our lab, and none had histologic evidence of damage.

In group 2 (1% phosphorus), only two of the six animals in the intact group had kidney calcium concentrations outside of the normal range, and only one of these animals had any histologic evidence of damage (Fig. 4). In contrast, all but one kidney from the partial nephrectomized and uninephrectomized groups contained increased calcium concentration, and all five animals in the uninephrectomized group with elevated kidney calcium concentrations had histologic evidence of disease. Because of the lack of a normal distribution of the data, the difference in calcification between the intact group and the groups with renal mass removed was not significant with standard statistical methods. Significance was achieved, however, with the Wilcoxon rank sum method for nonparametric data ($P < 0.05$) or by comparing $\log_{10}$ of the calcium concentrations ($P <$

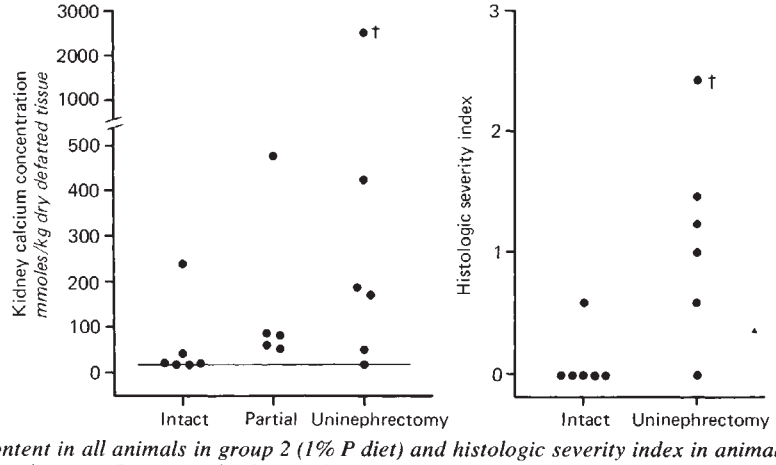

Fig. 4. *Kidney calcium content in all animals in group 2 (1% P diet) and histologic severity index in animals from subgroups A and C.* Only five animals are shown in group B as a result of one animal escaping from his metabolic cage prior to completion of the study. The symbol † indicates the single animal with an elevated plasma creatinine concentration.

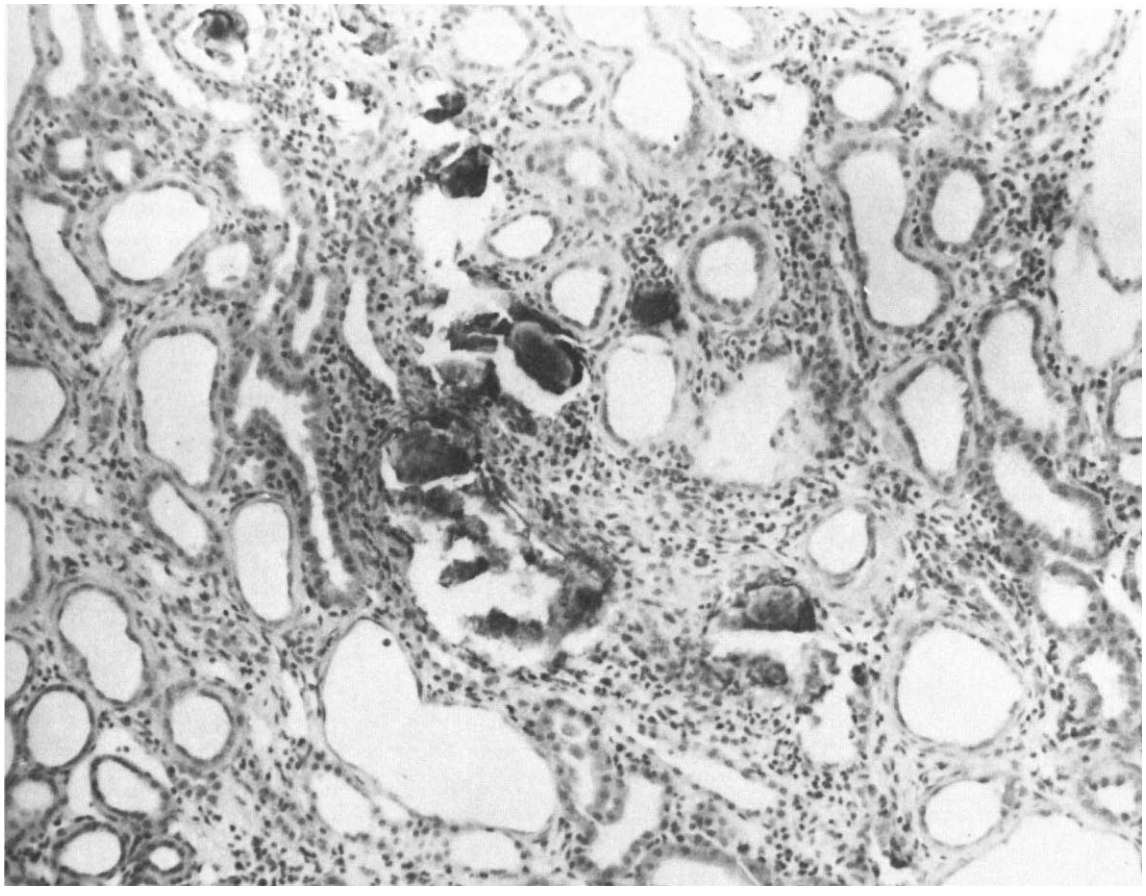

Fig. 5. *Histology of a kidney obtained from an animal in group 3C, demonstrating calcium phosphate deposit, interstitial fibrosis, tubular dilatation, and inflammatory cell infiltration.*

0.05). In addition, the histologic severity index was significantly higher in the uninephrectomized animals of group 2 as compared to the intact animals in that group ($P < 0.025$) (Fig. 4).

All animals in group 3 (2% phosphorus diet) had marked histologic evidence of disease and increased kidney calcium content. The uninephrectomized and partially nephrectomized animals on the 2% diet had significantly more calcification ($P < 0.01$ and < 0.025, respectively) than did the intact animals on the 2% diet. In addition, the uninephrectomized animals on this diet had a significantly higher histologic severity index ($P < 0.001$) than did the intact animals on this diet. Kidney phosphorus concentration rose in association with kidney calcium, supporting the deposition of a calcium phosphate compound (Table 2).

The histologic damage was limited to the interstitium and tubules. At the high phosphate loads per nephron, there was marked interstitial edema, fibrosis, and mononuclear cell infiltration. There was also tubular atrophy and dilatation. Calcification was most prominent in tubular lumens in the vicinity of the corticomedullary junction. There was some calcium in the interstitium, although much of the interstitial calcification could have originated in the tubules that subsequently degenerated. In some severely affected tubules, there appeared to be calcification of the tubular epithelial cells. An example of the histologic damage is shown in Fig. 5, which is from a uninephrectomized animal on a 2% phosphorus intake.

The histologic severity index and the kidney calcium concentration correlated extremely well for all animals in groups A and C ($r = 0.84$) ($P < 0.01$).

There was also a good correlation between net kidney weight and kidney calcium concentrations for animals in each subgroup at all dietary levels of phosphorus; subgroup A (intact), $r = 0.87$; subgroup B (partial nephrectomy), $r = 0.86$; subgroup C (uninephrectomy), $r = 0.88$.

There was a highly significant correlation ($r = 0.87, P < 0.01$) between phosphate load excreted per nephron and histologic severity index (Fig. 6).

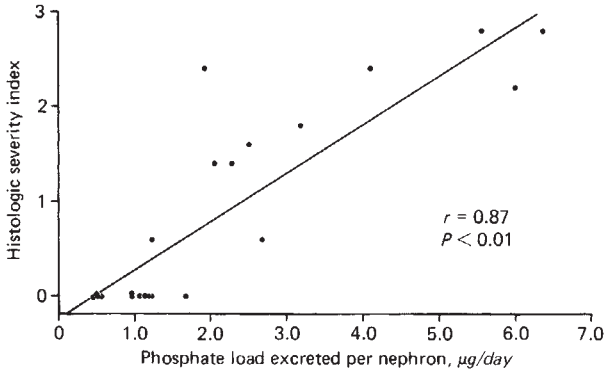

Fig. 6. *Correlation between phosphate load excreted per nephron measured at the week 7 and histologic severity index determined at the conclusion of the study (week 18).*

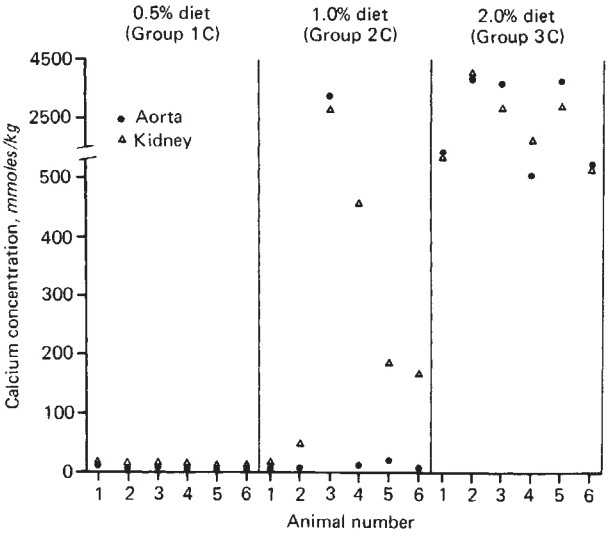

Fig. 7. *Calcium concentrations in aorta and kidney from all uninephrectomized animals in the three groups.*

Calcification of the aorta did not occur unless the animal had marked renal impairment, with elevation of the calcium-times-phosphorus product. In contrast, the kidney calcified in the absence of uremia at a time when the calcium-times-phosphorus product was normal. This is demonstrated in Fig. 7, in which the aortic and kidney calcium concentrations of the uninephrectomized groups on all three diets are shown. Four of six animals on 1% phosphorus had elevated kidney calcium concentrations with either normal or minimally elevated aortic calcium concentrations. The one uremic animal in this group had markedly elevated calcium concentrations in both organs. To exclude the possibility that a modest rise in plasma phosphorus levels and functional deterioration were responsible for some of the changes noted, we killed 18 animals after they received the phosphorus-supplemented diet for only 10 weeks. The six intact animals maintained on a 1% phosphorus diet had no renal histologic changes, and kidney calcium concentration (12.7 ± 0.41 mmoles/kg of dry wt) was within the normal range. In contrast, uninephrectomized animals on a 1% phosphorus diet had a kidney calcium concentration of 80 ± 34 mmoles/kg and a histologic severity index of 0.70 ± 0.22, and intact animals on a 2% phosphorus diet had a kidney calcium concentration of 557 ± 110 and a histologic severity index of 1.8 ± 0.17. No animal in either of these groups was uremic or had elevated plasma phosphorus concentrations at any time during the 10-week study. The final plasma phosphorus concentration was 6.27 ± 0.29 and 5.55 ± 0.20 mg/dl, and creatinine was 0.59 ± 0.02 and 0.52 ± 0.02, respectively, in these two groups of animals.

EHDP. Table 3 shows the mean values for weights, creatinine clearance per 100 g body wt, and urinary phosphorus excretion at 1 week and at 5 weeks into the study. In addition, plasma phosphate concentrations are shown at 1 week, 3 weeks, and 5 weeks. There was no significant difference between the groups in animal weights, creatinine clearance per 100 g of body wt, or urinary phosphorus excre-

Table 3. Diphosphonate (EHDP) balance data[a]

Time	Group	Weight *g*	C_{Cr} *ml/min/100 g body wt*	Urine P *mg/day*	P_P *mg/dl*
1 week	A (3% P + EHDP)	308 ± 12	0.42 ± 0.03	132 ± 9	7.28 ± 0.42
	B (3% P)	299 ± 5	0.42 ± 0.07	142 ± 4	9.24 ± 0.98
	P	NS	NS	NS	NS
3 weeks	A	336 ± 14	—	—	9.04 ± 0.71
	B	331 ± 14	—	—	9.60 ± 1.02
	P	NS			NS
5 weeks	A	333 ± 16	0.49 ± 0.06	206 ± 26	7.72 ± 0.35
	B	340 ± 15	0.47 ± 0.03	192 ± 20	11.1 ± 1.12
	P	NS	NS	NS	< 0.025

[a] Values are the means ± SEM. Diphosphonate is disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP).

Table 4. Diphosphonate (EHDP) histologic and analytical data

Group	Animal no.	Wet kidney wt *g*	Histologic severity index	Quantative calcification *mmoles/kg DDFT*[a]	S_{Cr} *mg/dl*
A (3% P + EHDP)	1	2.04	0.8	237	0.45
	2	1.61	0.8	83	0.45
	3	2.16	1.0	266	0.50
	4	1.63	0.2	116	0.50
	5	2.08	0.8	85	0.40
Mean ± SEM		1.90 ± 0.12	0.72 ± 0.14	157 ± 39	0.46 ± 0.02
B (3% P, no EHDP)	1	2.71	2.2	489	0.95
	2	2.82	2.0	303	0.50
	3	2.88	2.6	384	0.85
	4	2.47	2.0	367	0.45
	5	2.57	1.4	151	0.45
Mean ± SEM		2.69 ± 0.08	2.04 ± 0.19	339 ± 56	0.64 ± 0.11
P		<0.001	<0.001	<0.05	NS

[a] DDFT is dry defatted tissue.

tion. The mean plasma phosphate concentration was significantly higher in the group not on EHDP only at the 5-week sample ($P < 0.025$).

Table 4 shows data obtained at the end of the study (6 weeks). Group B, which did not receive EHDP, had a significantly higher wet kidney weight ($P < 0.001$), a significantly higher histologic severity index ($P < 0.001$), and a significantly higher kidney calcium content ($P < 0.05$). The creatinine concentrations were not significantly different between the groups, but the two animals with the highest histologic severity index (B1 and B3) had creatinine values above the range of normal for our lab. There was excellent correlation ($r = 0.83$) between the calcium concentration of the kidneys and the histologic severity index.

Discussion

This study confirms earlier reports showing that extremely high phosphate diets cause renal disease in normal animals [1–4]. It is also apparent, however, that as renal mass is reduced, renal injury is intensified, and less phosphate intake is required to induce renal injury. Animals receiving a 2% phosphorus diet with both kidneys intact have no change in renal function, whereas uninephrectomized and partial nephrectomized animals on a similar diet undergo a marked functional deterioration during the study period. Furthermore, in animals with only one kidney, a phosphorus intake two times normal for the rat (1% phosphorus) causes marked calcification and histologic changes in the remaining kidney, whereas animals with two kidneys tolerate this amount of phosphorus reasonably well. These studies further suggest that renal injury is directly a consequence of the intrarenal phosphate load, in that animals with one kidney on a 1% phosphorus diet who have marked histologic alterations in the kidney have plasma phosphorus concentrations similar to uninephrectomized animals on a 0.5% diet who have no renal injury. In fact, even uninephrectomized animals on 2% phosphorus diets tend to have normal phosphorus concentrations until functional impairment occurs. In addition, animals killed at 10 weeks, prior to the time that plasma phosphorus increases in any group, also have extensive renal calcification and histologic changes. Thus, it would appear that the nephrotoxicity of phosphorus is markedly increased as renal functional mass is decreased. This is graphically displayed in Fig. 8. In animals with 100% of renal mass, toxicity occurs when phosphorus intake is increased four times normal, whereas animals with 50% reduction in mass develop a comparable degree of toxicity when dietary phosphorus is increased only twofold. With further reduction in renal mass, as found in the remnant kidney model, toxicity occurs at normal phosphorus intake [9]. These observations may explain the progressive functional deterioration that occurs when a critical amount of renal tissue is removed in the rat and possibly other species [17–19]. In support of this is the fact that functional deterioration in rats with a remnant kidney can be prevented by phosphorus restriction [9].

In the present study, as well as in animals with the renal remnant, there are increased GFR and filtered load of phosphorus per nephron. In experimental glomerulonephritis, however, where single nephron GFR and filtered load of phosphorus may be normal or reduced, kidney calcification also oc-

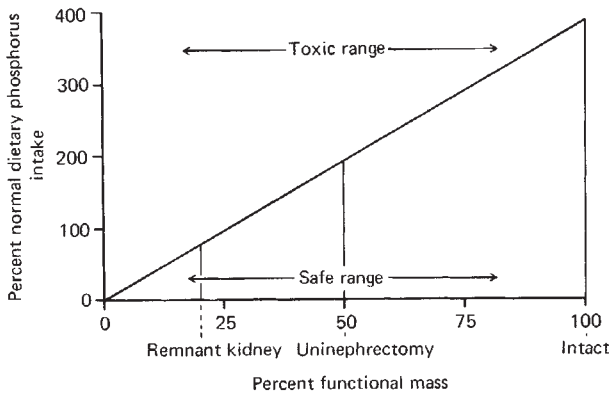

Fig. 8. *Renal toxicity of phosphate in regards to dietary intake and amount of functional renal tissue.*

curs [10]. This suggests that an increased filtered load of phosphorus may not be the entire mechanism responsible for renal calcification.

It has been shown that during chronic oral phosphate loading there is a marked adaptation, only partially mediated through parathyroid hormone, for increasing phosphorus excretion [11-13]. In addition, in the adaptive state, tubular secretion of phosphorus has been shown [20]. Thus, it may be that these adaptive mechanisms that would be expected to be present in renal failure as well as during phosphate loading could be injurious to the kidney, promoting calcium phosphate deposition, which in turn incites an inflammatory and fibrotic response.

These studies would further support the contention that phosphate-induced renal injury is mediated through calcification. First, there is a strong correlation between the degree of calcification and severity of histologic injury ($r = 0.84$). Second, animals given EHDP have significantly less renal histologic injury as compared with animals receiving a comparable phosphate intake without EHDP. EHDP could affect calcium phosphate deposits in two ways. First, it would tend to prevent the formation of apatite or brushite [21-23]; and second, it would prevent the transformation of amorphous deposits to crystalline deposits [24]. The most apparent reason that EHDP has a protective effect is that it reduces the intensity of renal calcification.

Because the animals not receiving EHDP have significantly higher plasma phosphorus concentrations, it seems possible that this might also contribute to the increased kidney calcium content. But, as stated above, in the other groups of animals, kidney calcium content bears no relationship to plasma phosphorus concentrations.

The mechanism responsible for the phosphate-induced intrarenal calcification is not readily apparent. High intratubular phosphate concentrations could result in precipitation of calcium phosphate in the tubular lumen, possibly in association with change in tubular fluid pH. Second, MacKay and Oliver have suggested that high phosphorus loads are a direct toxin to the renal tubular cells [1]. In turn, dystrophic calcification could occur as a result of cellular injury. PTH could also play a role in the pathogenesis of the renal calcification, because it would be expected that animals on high phosphate diets would have elevated PTH levels, and PTH has been implicated in other forms of extraskeletal calcifications [25]. A fourth possibility, metastatic calcification, as stated above, seems unlikely because serum phosphorus concentrations bear no relationship to kidney calcium content. In contrast to kidney tissue, other soft tissues (aortic) did not undergo calcification until the animal became uremic and had marked hyperphosphatemia. Also, the animals killed at 10 weeks, prior to having any elevation in plasma phosphorus concentrations, have extensive renal calcification and histologic alterations.

We conclude that as renal functional mass is reduced, the renal toxicity of phosphorus is enhanced. Toxicity of phosphorus appears to be largely mediated through calcification. Until more is known in regards to phosphate toxicity in other species, it seems prudent to use phosphorus therapy with extreme caution in patients with even modestly reduced renal function. In addition, these observations may prove relevant to chronic forms of renal disease in which phosphate restriction may slow functional deterioration.

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